VISUAL PIGMENTS IN MAN

Rushton

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VISUAL PIGMENTS IN MAN

THE SHERRINGTON LECTURES

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VI *Visual Pigments in Man*, by W. A. H. Rushton, SC.D., F.R.S., Reader in Physiology in the University of Cambridge.

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VI

VISUAL PIGMENTS IN MAN

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CONTENTS

	List of Illustrations	vi
	Preface	ix
I	The Significance of Rhodopsin	1
II	The Measurement of Rhodopsin in Man	8
III	Cone Pigments and Colour Blindness	19
IV	Pigments and Colour Vision	27
	Bibliography	37

ILLUSTRATIONS

Figures and Illustrations in the text

Fig. 1 Drawing of the microscopic structure of the human retina showing rods *b* and cones *c* (Schultze 1866). 4

Fig. 2 Circles, the spectral sensitivity of the dark-adapted human eye (Crawford 1949). Curve, the absorption spectrum of human rhodopsin (Crescitelli & Dartnall 1953). 5

Fig. 3 Human dark-adaptation curve; log threshold intensity plotted against time in the dark after extinction of a bright adapting light (Kohlrausch, A., 1931. Handb. d. norm. u. path. Physiol. *12/2*, p. 1499). 7

Fig. 4 Diagram of headlamp reflexion from a cat's eye. R. retina; T. Tapetum lucidum (drawn by E.N.W.). 9

Fig. 5 Stiles' double monochromator used as retinal densitometer. (Rushton 1956). 10

Fig. 6 Measurement of rhodopsin in human eye during bleaching (white circles) under three levels of illumination (1 unit=20,000 trolands). Black circles show the subsequent regeneration in the dark (Campbell & Rushton 1955). 11

Fig. 7 Diagram of arrangement of area of retina bleached (white circle) and measured (shaded circle). In (A) the measured area was bleached; in (B) a neighbouring area was bleached. 12

Fig. 8 The curve shows the difference spectrum of rhodopsin. Rectangles show the average (with standard deviations) of difference spectrum

measured by retinal densitometry. The horizontal dimension gives the wave bands of the measuring light. (Rushton 1956). 13

Fig. 9 The curve shows the theoretical relation between the log intensity of a 10 second bleaching exposure and the fraction of pigment bleached. Points show the experimental results. The curve has been slid sideways to position of best fit. (Rushton 1956). 14

Fig. 10 Curves show the rod density at various points on the retina from the histological study of Østerberg (1935). Circles show the relative rhodopsin concentration as measured at these points by retinal densitometry. (Campbell & Rushton 1955). 15

Fig. 11 Dark-adaptation curves after 2 minute exposures to retinal illuminations shown (1 photon=1 troland). Black points are where the violet flash appeared coloured (cone vision), white points where it appeared colourless (rods). (Hecht, Haig & Chase 1937). 16

Fig. 12 Cone pigment measured on the fovea of a colour-blind subject. Effect of bleaching and regeneration (Rushton: unpublished). 20

Fig. 13 Difference spectrum of the cone pigment in the protanope after half bleaching with red light (circles) or with green light (squares) or after a total bleach (lines which give the wave bands used). (Rushton: unpublished). 21

Fig. 14 Rectangles, the difference spectrum of the visual pigment of protanopes showing wave bands used and standard duration of results. The curve is the protanope luminosity curve. (Pitt, F.H.G., 1944). 22

Fig. 15 Squares show difference spectrum upon bleaching with a deep red light. Circles show total bleach scaled to coincide with squares at red end. Dots show difference between circles and squares, and coincide with the curve which is the curve of the protanope, Fig. 14. (Rushton: unpublished). 24

Fig. 16 Log spectral sensitivity curves plotted against wave frequency. Vertical lines: action spectral; A, chlorolabe, B, erythrolabe (Rushton). Circles: spectral sensitivity of the fovea of A, protanopes; B, deuteranopes (Willmer 1950). Crosses: artificial monochromacy, A, green; B, red (Brindley 1953). Dots: increment threshold mechanisms, A, the green mechanism π_4, B, the red mechanism π_5 and blue mechanisms π_1 and π_3. Curve: the blue mechanism calculated from colour matching. (Stiles 1953). 34

PREFACE

In doing me the great honour of appointing me sixth Sherrington Lecturer the University of Liverpool has in some ways broken with tradition. For they have turned from the succession of giants to a man of ordinary stature and bestowed this distinction upon one who was neither a colleague of Sherrington nor his pupil, nor is even a specialist as to brain or spinal cord.

Whatever may be thought of their choice of man, however, it cannot be doubted that the organisation of the retina is a most appropriate topic with which to commemorate what Sherrington stood for in scientific thought. For, as he clearly realized, the retina is a self-contained and exquisitely organized outgrowth of the central nervous system, and nowhere is the relation between neural interaction and sensory perception more clearly displayed. But the wider I paint the sweep of my subject, the narrower will appear the treatment I have given it.

During the last few years we have developed a technique for measuring visual pigments in the living eye, especially in the eye of man. My present intention is to place these new results (still very incomplete) into some perspective in relation to information and colour theory.

I

THE SIGNIFICANCE OF RHODOPSIN

1. *The Biological Basis of Visual Pigments*

We and other animals obtain nearly all our information about the outside world from receptors situated near the surface of the body. In the most primitive species the receptors respond to immediate contact such as touch and taste. But enormous rewards are offered in the struggle for existence to those with advanced information and few there are who can manage to survive without smell, hearing or sight—the Distance Receptors of Sherrington. For the alternative would be to starve, to be eaten, to become extremely unpalatable or to enjoy a rare fecundity in reproduction. Vision has great advantage over touch, for we can see far beyond our reach. Moreover, there is secrecy in sight, since it is much easier to see without being seen than to feel without being felt.

But the possession of distance receptors brings complication—the complexity of a nervous system capable of extracting what is important from a very mixed message and of organizing appropriate responses. If our skin is irritated by the arrival of an insect we scratch the place, the irritation ceases, the insect is removed and the response is clearly appropriate. If, on the other hand, our retina is irritated by the arrival of an income tax demand, we may indeed scratch out our eyes and so remove the immediate irritation but this response is not appropriate because the tax demand persists and, as is well known, it requires a far more ingenious behaviour pattern for successful evasion. Thus it is no coincidence that nerves from eyes, ears and nose run directly into the brain, for the brain is largely the elaboration of central nervous tissue which is required to process properly the input patterns from the distance receptors.

The receptor which forms the subject of this Lecture is the eye—that superb trick of adaptation which Nature has brought off

again and again in the course of evolution. We know how to touch, how can we manage to see? Light travels in straight lines, so the position of objects around us may be defined by the direction of the light coming from them, and this direction may be recorded either by an image formed by the lens system of vertebrates and cepahlopods or in the omatidial system of the compound eye of arthropods. In each case a replica of the luminous world outside is formed within the body, and thus by "feeling" this replica we may embrace the stars.

But how may something so intangible as an optical image be felt? Light it is that forms the image, so light must do whatever is done when the image is felt. And the only thing that light can do is to react with matter. That is a quantum event.

In the same way that matter which seems continuous is in fact composed of discrete atoms, so light will only react with matter in discrete "atoms" of energy called *quanta*. A beam of light then may be regarded as a shower of quanta, and when we look at Jupiter he appears to us as he did to the mother of Perseus in the old Greek legend, as a golden shower. Danae, you may remember, was shut in a tower of brass, and we are not told what she did with her gold, but she may well have used it as we do—imprisoned as we are in a few feet of flesh—to buy *information* about what is going on in the world outside us. But before we can barter our quanta we must first catch them, and since anything which catches a light quantum is called a pigment, the eye must contain a pigment.

Now the sort of pigments which first come to mind are those used to colour objects—*e.g.* paints and dyes. But these are precisely the wrong kind of pigment to form the starting point of vision. For our curtains must not fade in the sun nor our pictures blacken upon the wall. No chemical change should follow the absorption of light by good paints or dyes. Radiant energy should be dissipated in that gentle molecular vibration which constitutes a rise of temperature, without the violence of any chemical disruption. But light so utterly degraded is useless as the starting point of vision, for there is far more *heat* brought to the retina by one dark pulse of blood than in the whole bright conspectus of the Port of Liverpool. To initiate vision we need a pigment which is the reverse of stable—a powder magazine which will blow up upon receiving a direct hit from one quantum.

Molecules respond to light as do people to music. There are some who are unaffected and absorb nothing, there are some who react by the degraded vibration of foot or finger, but some there are who rise and dance and change partners. Such a dance of atoms was seen in 1876 when Franz Boll first described the bleaching of rhodopsin.

2. *The Physiology of Rhodopsin*

It was realized at once that rhodopsin had properties which would make it suitable as a quantum-catcher for vision—a visual pigment. But it is one thing to say "This could be a visual pigment," and quite another to be able to affirm (as now we can) "This *is* a visual pigment." By what steps have we been able to reach the second conclusion? If it is true that rhodopsin catches the quanta by means of which we see, a stringent conclusion follows. It is an application of Draper's Law. Not all wave lengths of light are equally absorbed by rhodopsin, and light which is not absorbed will not give up its energy, and hence will not be seen. We might therefore expect that wave lengths of light will be well or badly seen in proportion as they are well or badly absorbed by rhodopsin. Put more technically, the spectral absorption curve of rhodopsin should coincide with the spectral sensitivity curve of the eye when scaled to the same maximum.

Now it has been known for a very long time that the eye's spectral sensitivity is very different if measured in bright daylight or by faint moonlight. By day the maximum sensitivity is in the yellow-green (550 mμ for an equal quantum-energy spectrum), but in twilight it is in the blue-green (500 mμ). Which of these spectral sensitivity curves should we select to match with rhodopsin?

The answer to this comes in the first place from the classical study by Schultze (1866) upon the comparative histology of the retina in many species of vertebrate. He found in most eyes two types of photoreceptors, the rods and the cones, and in Fig. 1 we see his drawings from the human retina. In the various animals studied, Schultze found that the proportion of rods to cones differed considerably; in general those animals who have mainly *cones* are active only in daylight and at sundown go to ground or to roost, whereas night animals have predominantly *rods*. Upon

this classification mankind should be considered best suited for night life, for 95% of our retinal receptors are rods.

Kühne (1878) saw rhodopsin in the excised human retina, and saw that it lay in the rods and was absent from that little region, the *fovea centralis*, which contains only cones. This, then, gives the answer to our question as to which sensitivity curve is to be

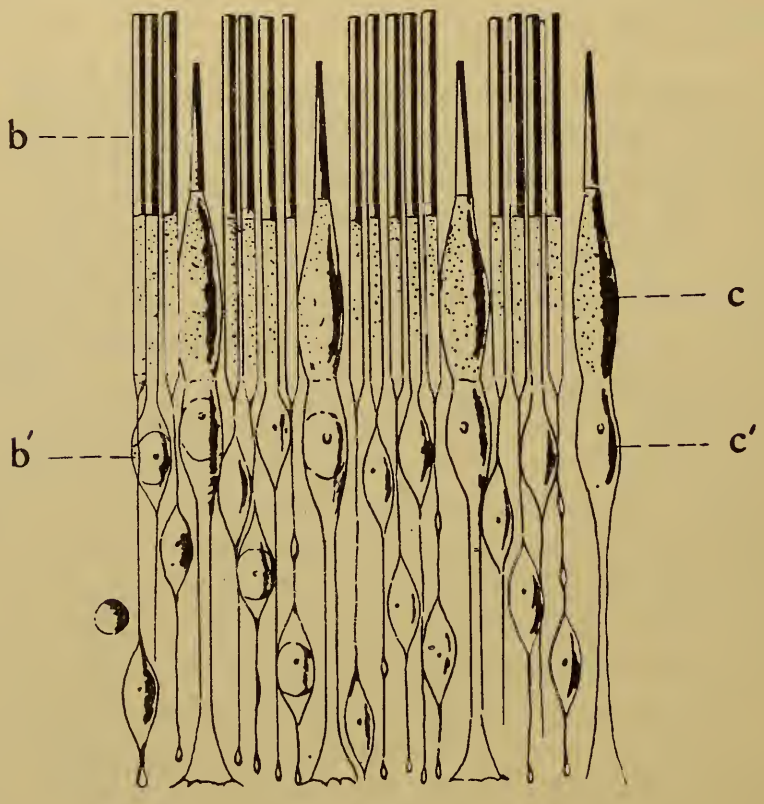

Fig. 1

Drawing of the microscopic structure of the human retina showing rods *b* and cones *c* (Schultze 1866).

compared with rhodopsin absorption. For rhodopsin is contained only in the rods, and they are responsible for twilight vision, so it will be the twilight or *scotopic* sensitivity curve which should be compared with rhodopsin absorption.

This comparison has been made in Fig. 2. A human eye had to be removed for a condition which did not affect the retina and with the co-operation of patient and surgeon the eye was previously kept covered, and excised by operating in red light, which is not appreciably absorbed by rhodopsin. The human rhodopsin

extracted (in the dark) from this eye had the absorption spectrum shown in the curve of Fig. 2 (Crescitelli and Dartnall). The circles give Crawford's (1949) average value for scotopic human visibility (corrected slightly for transmission losses in passage through the eye media, etc.). The coincidence of the two results is so striking that there can be no reasonable doubt that rhodopsin

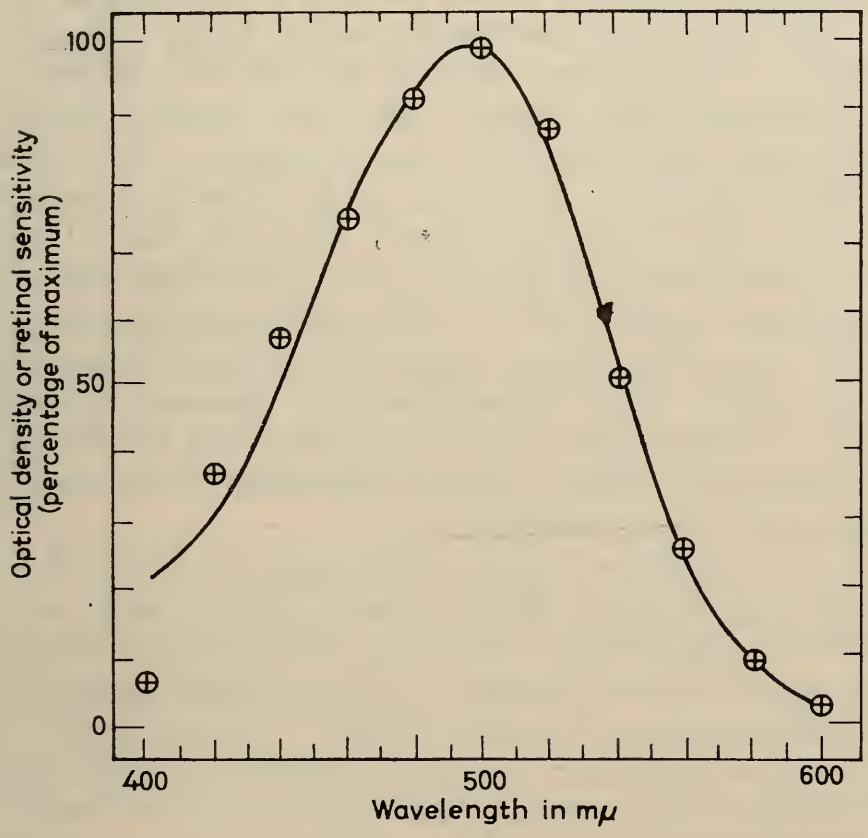

Fig. 2

Circles, the spectral sensitivity of the dark-adapted human eye (Crawford 1949). Curve, the absorption spectrum of human rhodopsin (Crescitelli & Dartnall 1953).

is the visual pigment used in twilight. It also strengthens Schultze's view that rods are the photoreceptors of night vision.

Sensory physiology is a study where the gap separating mind and matter is nearly bridged. An arch of hypothesis is built up from our sensations and reaches out towards the material shore whence the interlacing structure of pointer readings arches back. The correspondence of Fig. 2 drops like a keystone into this arch and cements the whole construction.

3. *Light Adaptation and the Bleaching of Rhodopsin*

The effect of light upon rhodopsin is to turn it into an almost transparent chemical, so the change is usually referred to as "bleaching." An excised retina exposed to strong light is soon quite bleached, but in the normal living eye this would not be expected to occur so fast or so completely, because the retina is in a dark chamber with only a small window for the admission of bright light, and because the bleaching is continually made good by a process of regeneration which, in the dark, fully restores the rhodopsin content to the retina. These two opposing processes, bleaching and regeneration, will cause rhodopsin to come to an equilibrum level which will be lower the brighter the maintained light. Now if less rhodopsin is present there will clearly be less chance of quanta being caught by it, so the incident light will have to be increased if quanta are to be absorbed at the same rate. If, therefore, the condition of just seeing in various states of adaptation were that a fixed number of quanta had to be absorbed, we should expect the visual threshold to rise during light which bleaches, and to fall during dark which permits regeneration. This certainly happens as may be seen from Fig. 3.

As is very well known, after a bright light the threshold (*i.e.* the faintest light intensity which can just be seen) is raised, and in the dark it then falls along a time course which plotted as in Fig. 3 shows a curve with two branches. In the figure the logarithm of the threshold intensity is plotted vertically. The first point was obtained immediately after a period of adaptation to bright light, and subsequent points represent the logarithm of the threshold after various times in the dark. The early branch of the curve must be due to cones since it may be obtained from the *fovea centralis* which contains no rods. The second branch on the other hand is not obtained on the fovea, and if the flashes are presented with lights of different wave lengths, they all appear the same green-gray colour and exhibit the spectral sensitivity of rhodopsin. This therefore represents rod vision, and is the part of the dark adaptation curve where the threshold would be expected to depend upon the amount of rhodopsin bleached if the quantum-catching power of the pigment is what in fact determines the threshold. Practically all text books state or suggest that this relation is true but give no evidence. That is because no evidence has ever been forthcoming.

In order to compare the threshold during dark adaptation with the rhodopsin content of the rods we may make various plausible assumptions. We may suppose that the relation between threshold and the electroretinogram (E.R.G.) in animals is the same as in man during dark adaptation, and compare the E.R.G. with the rhodopsin extracted from the eye at various stages of dark

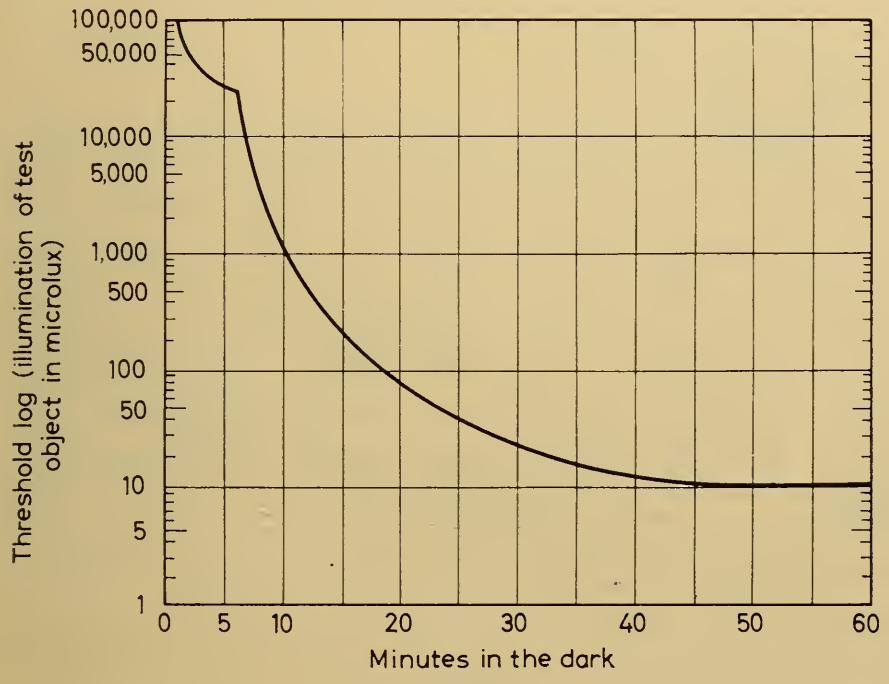

Fig. 3

Human dark-adaptation curve; log threshold intensity plotted against time in the dark after extinction of a bright adapting light. (Kohlrausch, A., 1931. Handb. d. norm. u. path. Physiol. *12/2*, p. 1499).

adaptation (Granit, Munsterhjelm & Zewi 1939). Or we may assume that rhodopsin molecules will not be bleached faster than quanta can enter the eye, and so obtain an upper limit to the amount of bleaching produced by a given light adaptation (Baumgardt 1949). It is very likely that these assumptions are sound and if so it is quite clear that the lowering of threshold during dark adaptation is *not* appreciably due to the improved quantum-catching power by the regeneration of rhodopsin. But the most direct way of answering our question is to measure the actual density of rhodopsin in the human eye during bleaching and regeneration. This has been done by Campbell and me.

II

THE MEASUREMENT OF RHODOPSIN IN MAN

1. *Method*

It sometimes happens when driving at night that the eye of a cat is caught in the headlight beam from the car, and the whole cat's eye is then seen full of light. The path of the rays is shown in Fig. 4 whence it appears that though only a small part of the retina is illuminated, reflexion from this region is rather precisely focussed back upon the light source and upon the driver's eye which lies just behind it. The cat's retina is fairly transparent and light is reflected from a mirror, the shining *tapetum lucidum* behind it. The light which emerges therefore has traversed the retina twice and suffered a double absorption by whatever retinal pigments are there. If this were the only absorption we should measure it at once by comparing the spectral content of ingoing and emergent light. But even in the cat the chief loss occurs at the reflecting surface behind the retina and this must be eliminated from our measurements. In principle this can easily be done by making use of the fact that exposure to a strong light will bleach rhodopsin but leave the rest of the system unaltered. The *change* in reflectivity of the eye therefore, will measure simply the change in the amount of rhodopsin present, and this measurement can be done even in the human eye, where there is no tapetum and reflectivity is very poor.

Now any method which assumes that a change in the amount of light received back from the eye is a measure of the change in visual pigment must be protected from light changes due to other things. For instance it is not easy to keep a light source absolutely steady in intensity, nor a delicate detector constant in sensitivity, and hardest of all, the human eye never keeps quite still. The ceaseless movement produces a fluctuation in the reflected light which makes accurate measurement hard. All these difficulties are largely overcome by sending into the eye along the same path not

one light beam but two of different colours, a red and a blue-green. As may be seen in Fig. 2 a blue-green light of wave length 500 mμ lies at the peak of the rhodopsin absorption curve, so the amount of this reflected from the eye will depend greatly upon the amount of rhodopsin there is to absorb it. Red light of wave length 620 mμ on the contrary is hardly absorbed at all, so the

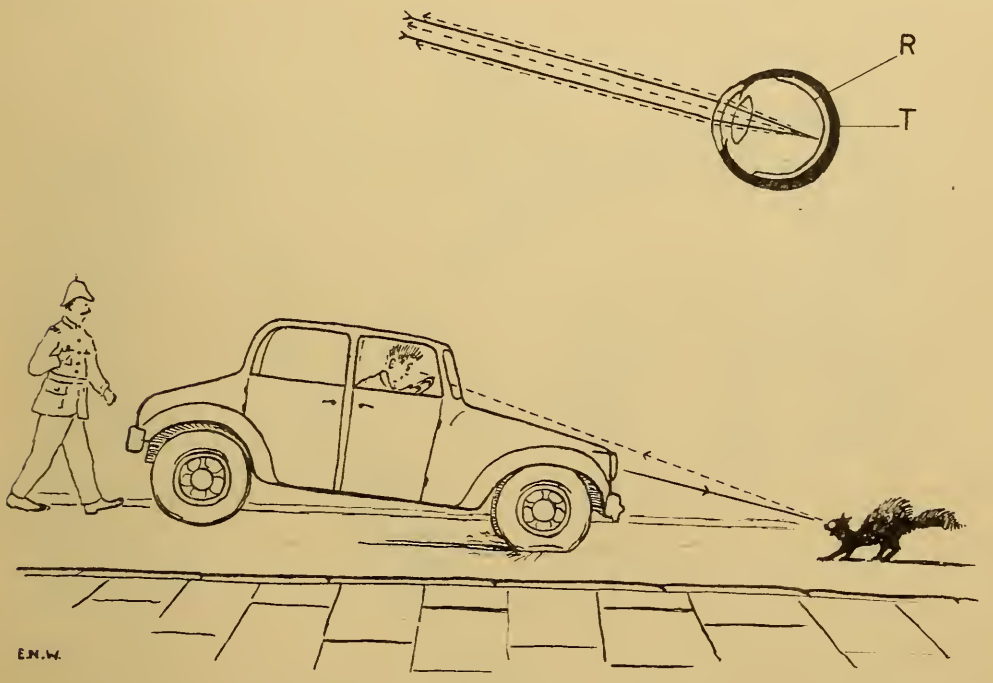

Fig. 4

Diagram of headlamp reflexion from a cat's eye. R. retina; T, tapetum lucidum (drawn by E. N. W.).

amount reflected will be independent of rhodopsin, but will be influenced by all the disturbing factors to the same extent as will blue-green. Disturbance may therefore be eliminated from the measurements as follows. The red and green lights are applied in rapid alternation, and the light reflected from the eye is received upon a very sensitive photomultiplier tube. The output current from the tube is sent through a galvanometer by way of a reversing switch which changes with change in colour of the light. Thus the green light deflects the galvanometer to the right, the red light to the left. The brightness of the red beam is controlled by a photometric wedge and the intensity is adjusted to bring the galvanometer deflection to a fine tremor about the zero point. Since disturbing factors affect both beams equally they will not

change the deflection from zero. But any increase in rhodopsin density will diminish the green output but not the red, and so cause a deflection. When zero has been restored by shifting the wedge, the increase in its density must exactly equal the increase in the density of rhodopsin, and thus the latter may be measured simply by reading the shift of the (calibrated) wedge. This in

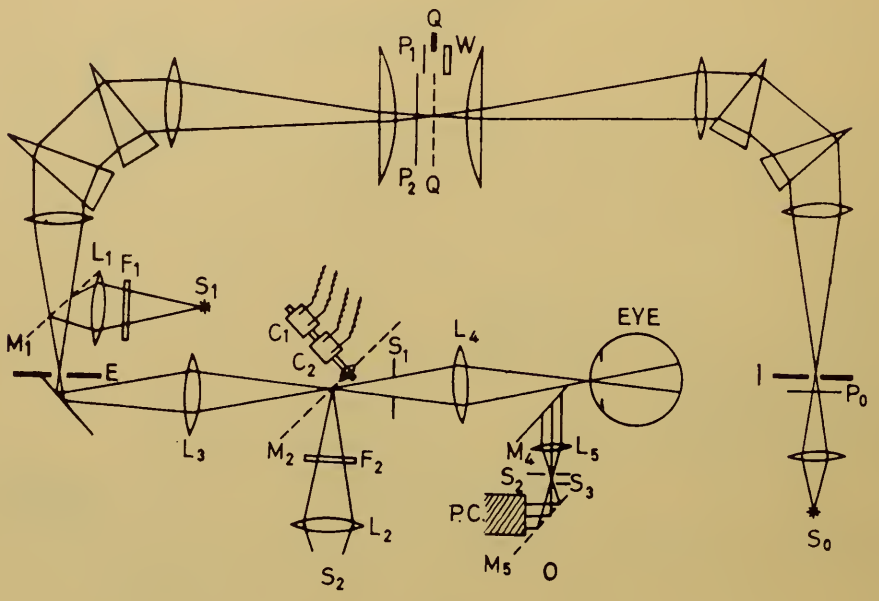

Fig. 5

Stiles' double monochromator used as retinal densitometer. (Rushton 1956).

principle is the method which Campbell and I first used (1955), and this also underlies the more complicated apparatus shown in Fig. 5. We cannot enter here into technical details of equipment or procedure, but a few features may just receive mention.

The head is immobilized by biting upon a dental impression and leaning against a forehead rest. The eye is kept steady by looking at a small fixation light, usually 15° from the direction of the measuring beam. The pupil is fully dilated with homatropine to permit maximum light flux. With a good subject measurements take 5-7 secs. to make and are immediately repeatable with an error of about 0.005 density unit; over an extended period the error may be twice that. The total (double) density change upon bleaching is about 0.15, so measurements may have an over-all reliability of 6% of the total content. The more elaborate

equipment of Fig. 5 (Rushton 1956) permits the pigment density to be measured in lights of various wave lengths (not only blue-green) by varying the position of the slot in the spectrum QQ. Moreover it is possible to make such measurements while bleaching is taking place from a different coloured light S_2, applied intermittently by rotating the sectored mirror M_2.

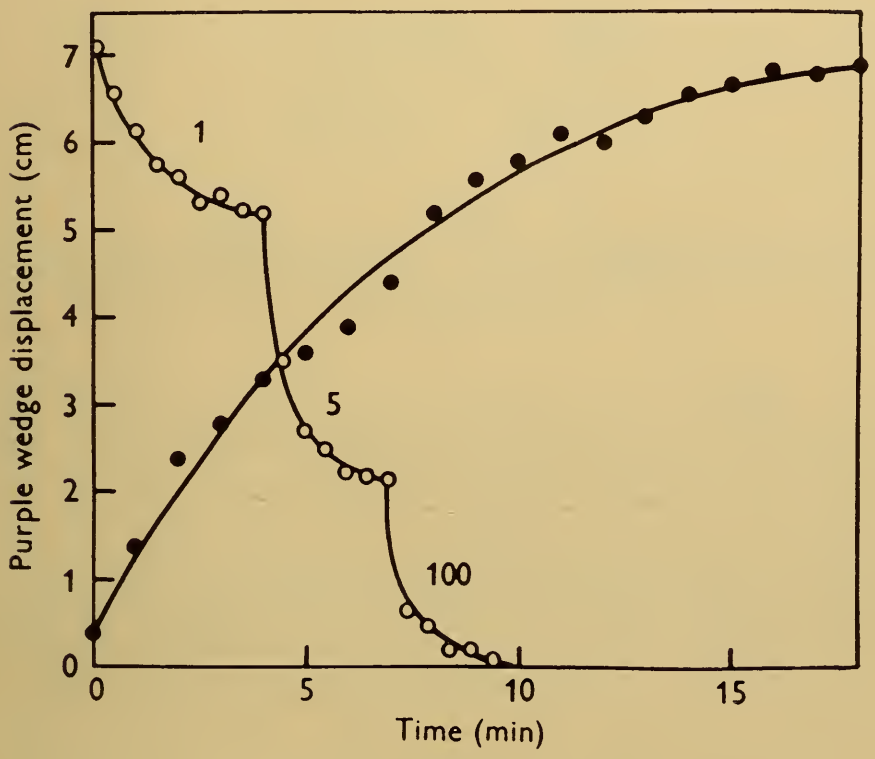

Fig. 6

Measurement of rhodopsin in human eye during bleaching (white circles) under three levels of illumination (1 unit = 20,000 trolands). Black circles show the subsequent regeneration in the dark (Campbell & Rushton 1955).

2. *Results*

Fig. 6 shows the change in my eye as measured by Dr. Campbell (1955). The region measured was about 15° from the fovea on the side away from the blind spot and starts after a period of 20 minutes dark adaptation. A bright light of 1 unit of intensity (20,000 trolands) is seen to bleach fast at first, then slower as would be expected if the pigment was nearly all removed or if the removal was being counterbalanced by regeneration. The first alternative is clearly false since increasing the light to 5 units

bleaches both faster and more extensively, coming to equilibrium at a lower level. Even this is not complete bleaching, but 100 units reaches a level which cannot be depressed much further. In the dark (black circles) regeneration occurs, fast at first, later slower, and in about 20 minutes it reaches the initial level (which was in fact the state after 20 minutes dark adaptation).

Fig. 7

Diagram of arrangement of area of retina bleached (white circle) and measured (shaded circle). In (A) the measured area was bleached; in (B) a neighbouring area was bleached.

These results are very much the sort of thing we should expect to find if we were measuring rhodopsin, but you may well consider it such a tricky business to determine the amount of a visual pigment from tiny changes in a very feeble light, that you would like a bit more assurance that rhodopsin is what in fact is being measured. We have tried to obtain evidence upon this point by a number of approaches which may briefly be quoted.

(a) Fig. 7 (A) represents by the shaded circle the small 3° area of retina whose rhodopsin density was studied. The bleaching light was normally directed so as to include this area, as shown by the larger circle. If now the bleaching light fell just clear of the measuring area as in Fig. 7 (B), it was found to produce no measurable bleaching even though the intensity was 100 times that which would bleach in condition (A). Thus what is measured is certainly a change *localized* to the bleached region of retina: it could not be a general change such as of pupil size or lens focus (though in fact the homatropine used to dilate the pupil abolished both of these actions).

(b) We have seen (Fig. 2) that lights which look equally bright by scotopic vision are equally absorbed by rhodopsin. They should therefore all bleach equally, and produce equal changes in wedge setting if we are in fact measuring rhodopsin. I adjusted lights of various wave bands so that they all appeared equally

bright to my twilight vision. These lights were far too weak to bleach appreciably so they were all increased by exactly the same factor (of about 10,000). Then Dr. Campbell bleached my eye with each in succession and every one produced the same change of wedge setting, which corresponded to about 50% of the total bleach. This result means that what we measured was a change

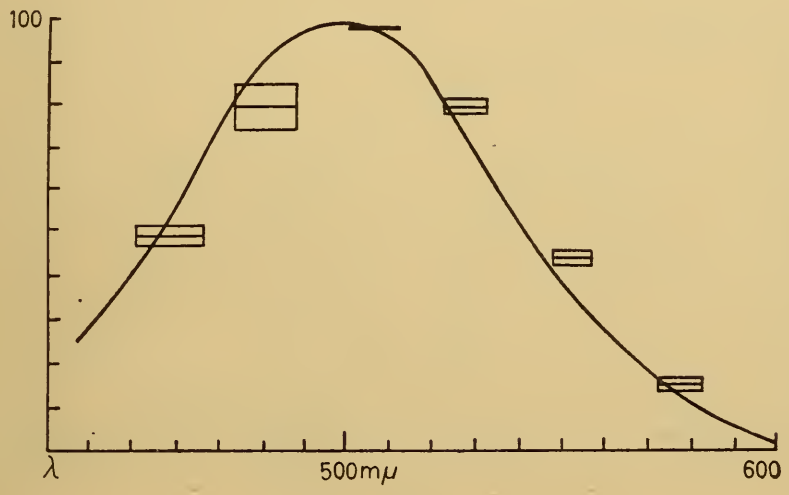

Fig. 8

The curve shows the difference spectrum of rhodopsin. Rectangles show the average (with standard deviations) of difference spectrum measured by retinal densitometry. The horizontal dimension gives the wave bands of the measuring light. (Rushton 1956).

which originated in the bleaching of rhodopsin, but it was not necessarily *the bleaching* itself that was the change measured. For instance any reflex initiated by rods only would exhibit this relation. But a reflex would not be likely to give the result now to be described.

(c) The Bunsen-Roscoe Law (Intensity × time = constant for a fixed photochemical change) should hold without limit for a photochemical reaction where regeneration does not occur. We found it to hold up to 45 sec. as judged by a fixed change in wedge setting. This is what would be expected if bleaching was accompanied by simultaneous regeneration occuring at the rate shown by the black circles of Fig. 6. But the *It* relation involved in visual *reflexes* is at least 50 times quicker than this—the appearance of a dim light lasting 30 sec. is obviously quite different

from one 15 times as strong lasting 2 sec. So our measurements depend upon chemical not nervous events.

(d) If we measure the change of reflectivity in a total bleach using various wave bands (not just 500 mμ) for our measurements, the wedge shifts plotted against wave length should give the difference spectrum of rhodopsin (the change in absorption upon

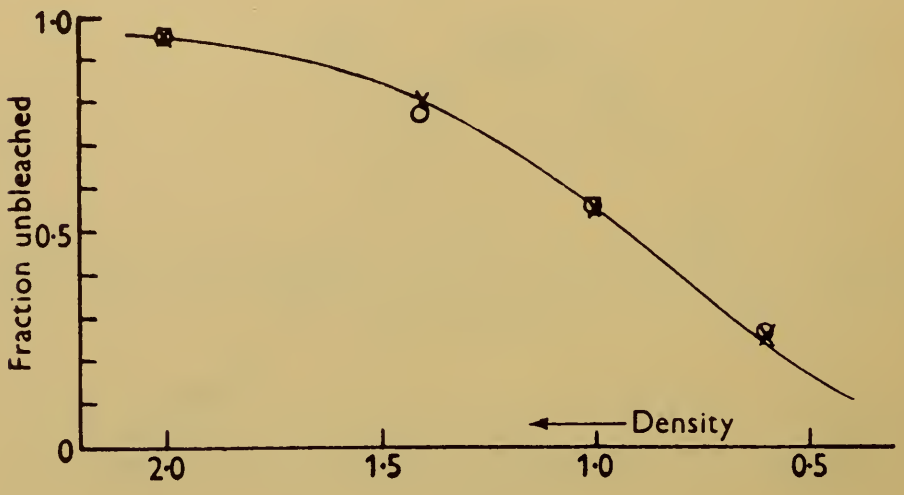

Fig. 9

The curve shows the theoretical relation between the log intensity of a 10 sec. bleaching exposure and the fraction of pigment bleached. Points show the experimental results. The curve has been slid sideways to position of best fit. (Rushton 1956).

bleaching). Fig. 8 shows the expected curve and the experimental rectangles which lie reasonably close to it. So we measure something with spectral absorption similar to rhodopsin.

(e) The physical chemistry of photolysis allows one to work out the relation between the intensity of a bleaching flash and the amount of pigment bleached. This curve is shown in Fig. 9 and the experimental results are given by the points. The curve has one arbitrary constant and may be slid horizontally along the scale of log I to fit the points. The good fit confirms that we measure the bleaching of a photosensitive pigment, and the actual position of the curve indicates that the pigment is bleached twice as fast as would be a solution of rhodopsin. Now the outer segments of the rods (the free ends Fig 1) which contain the rhodopsin are smaller than the inner segments, which first receive the light and appear to funnel it into the outer regions as indeed

Schultze noticed. This would increase the light intensity upon the rhodopsin and might well account for the increased bleaching rate which is of about the right order of magnitude.

(f) If rods were evenly distributed throughout the retina we should expect the same wedge change following a total bleach wherever we measured it. But it is known that distribution is

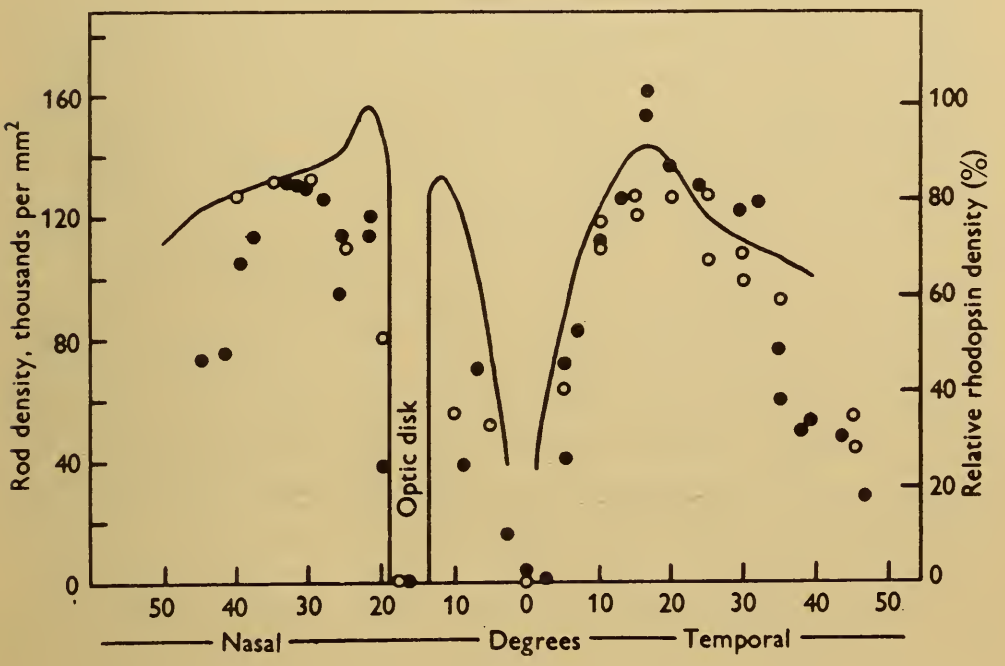

Fig. 10

Curves show the rod density at various points on the retina from the histological study of Østerberg (1935). Circles show the relative rhodopsin concentration as measured at these points by retinal densitometry. (Campbell & Rushton 1955).

far from uniform and the curve of Fig. 10 shows the rod density at various points along the horizontal meridian (Østerberg 1935). At the fovea (0°) there are no rods, nor are there any upon the blind spot (15° nasal) and there is a maximal density at ± 20°. The black and white circles give the rhodopsin density at corresponding points (2 methods) and it is seen that these go hand-in-hand with rod concentration. In particular there is no rhodopsin on fovea or blind spot and at ± 20° it is maximal.

We may therefore conclude that what we measure is rhodopsin. For the change we observe is *localized* to the region of the retina bleached (a) above, and its magnitude is unaltered if the bleaching

energy *It* is kept the same for a bleaching time as long as 45 sec. (c). This points to a local chemical rather than a reflex change, and this is confirmed by the kinetics of bleaching (e) where the absolute value of the photosensitivity indicates rhodopsin. The action spectrum (b) and the difference spectrum (d) both correspond to rhodopsin and the distribution in the retina goes hand-in-hand with the density of rods (which contain rhodopsin), showing total absence at the two retinal sites where rods are absent.

The technique just described allows us to say with some precision what fraction of rhodopsin is bleached by any given bleaching light and how much has been regenerated after defined conditions of dark adaptation. For instance, it appears from Fig. 6 that about 2 units of bleaching light will come to equilibrium at 50% total bleach. This intensity is 40,000 trolands which corresponds to about 10,000 millilamberts viewed with a natural pupil. It is as bright a light as one will meet on a sunny day short of looking at the sun itself. So in our ordinary experience rhodopsin is never more than half bleached, and usually very much less so, as Kühne already stated for the frog, 70 years back.

Half bleaching will remove half the quantum-catching power of the pigment and so the threshold must be doubled (+ 0.3 log

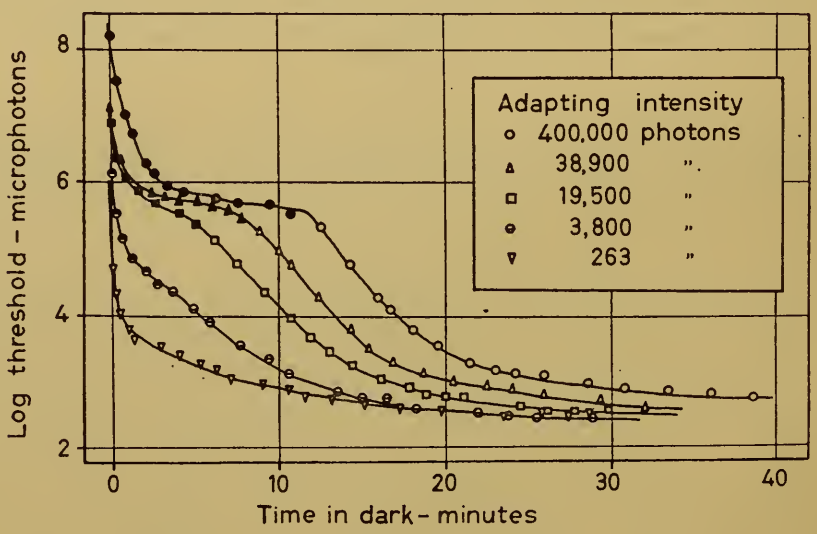

Fig. 11

Dark-adaptation curves after 2 min. exposures to retinal illuminations shown (1 photon = 1 troland). Black points are where the violet flash appeared coloured (cone vision) white points where it appeared colourless (rods). (Hecht, Haig & Chase 1937).

unit) from this cause. But after such a light adaptation, as may be seen from the results of Hecht, Haig & Chase (1937) shown in Fig. 11, rods (white triangles point up) not only have their thresholds raised enormously but they are completely removed from observable activity, and only appear after 9 minutes with a threshold still 1,000 times the final value. But as appears from Fig. 6, regeneration for 9 minutes from the point when the pigment was 50% bleached brings us to 90% of the complete rhodopsin complement. So we shall not be far wrong in saying that our rods are inoperative when they contain less than about 90% of their total rhodopsin content. The diminished quantum catching power due to bleached rhodopsin therefore will only account for about 0.0001 of the observed threshold change.

3. *Threshold and bleaching*

This is an awkward conclusion and we can't help but resent it. We are blind for quite a long time after going from light into dimness, and we don't understand why that should be. If practically all the rhodopsin had been removed, we should have some sympathy with the few molecules that remain and do what they can to catch and use the feeble light. But all the rods are packed full of rhodopsin and catch quanta as fast as they do in the dark adapted state. They simply won't use properly more than a few percent of the quanta they catch—it looks very like sabotage! I wish that I could reassure you upon the goodwill of the rhodopsin molecules. Some case can indeed be made but it will not go far towards satisfying us.

A retinal signal contains information in three categories, spatial discrimination (acuity), temporal discrimination (flicker fusion frequency) and reliability of the message (signal/noise discrimination). In principle, if we wish to increase the total information content of the signal, we must catch more quanta, but for a fixed quantal catch, information may be diverted into one or other of the three categories at the expense of the others.

For instance Pirenne & Denton (1952) have suggested that the improved acuity which is experienced as illumination increases is because smaller clusters of rods are now signalling, so there is a finer grain to the retina. But small clusters cannot catch as many quanta as large clusters so their absolute threshold will be higher. A nerve organization, therefore, which broke up large rod

clusters into small ones would lead to improved acuity in the light and to raised thresholds on going into the dark. And the same kind of argument might be made with regard to temporal resolution.

Though nerve organization plays some part in the threshold changes of dark adaptation there are two considerations which weigh heavily against its role being anything but a minor one.

First, the range illuminations over which rod discrimination improves is entirely too feeble to produce appreciable bleaching. On the other hand bleaching lights which produce the marked rise of thresholds are very far above cone threshold and quite out of the range where reorganization of rod clusters would have any purpose since, as Aguilar & Stiles (1954) have shown, the rods are saturated and can no longer discriminate anything.

Second, the dark adaptation curve seems to be linked with the regeneration of rhodopsin, for with cones and rods in man and some animals the sensitivity of the receptor continues to improve in the dark so long as the visual pigment is regenerating and then stops. This strongly points to the change of sensitivity residing in the photoreceptors where the pigment is, rather than in the organization of the nerve connexions.

At the present time I cannot see anything but disadvantage and waste in what the light-adapted rods do with most of their absorbed quanta. But bleaching is rough treatment. The rod is a photoreceptor which has achieved the perfection of sensitivity; it will respond to the ultimate minuteness of light energy—one quantum. Most instruments which are extremely sensitive have to be protected from excessive overloads, otherwise they are permanently damaged, or at least thrown off their working range for a time.

We know nothing of the mechanism by which the rods either transduce the light, or signal the resulting message, but it seems to depend in some way upon the ordered array of rhodopsin molecules in the quasi-crystalline structure of the rod. An occasional gap in the ranks of molecules seems to disorganize the whole parade ground, but whether this is the partial disruption of a fine mechanism or the purposive protection against its destruction by overload, we cannot say.

The fact is that removal of some 10% of the molecules will incapacitate rods to the verge of blindness.

III

CONE PIGMENTS AND COLOUR BLINDNESS

1. *Measurement of cone pigments*

Cones are the photoreceptors of daylight vision, the receptors of precision and of colour. Great interest therefore attaches to the study of the visual pigments which they contain. This has naturally been attempted by the methods which succeed so well with rhodopsin, namely by extracting the pigment from the photoreceptors in a solution of bile salts or digitonin. But extraction has proved so tricky that only Wald, Brown and Smith (1955) have ever been able to make a proper study of a cone pigment in solution. For this they chose to investigate the retinas of fowls since (as Schultze found in 1866) these birds have predominantly cone eyes, and in fact the cone/rod ratio is 1000 times that of man. Even so they found their extract to contain twice as much rhodopsin as cone pigment. It is thus clear that cones contain very little indeed of extractable visual pigment and any attempt to make a similar extract from the whole human retina would hardly yield 0.1% of cone pigment in 99.9% of rhodopsin.

It turns out, however, that measurement by retinal densitometry in the living human eye gives more favourable results.

It has long been known that cones are the only photoreceptors upon the *fovea centralis*—that precious central $\frac{1}{3}$ mm^2 of retina used for reading and all accurate observation—and Fig. 10 shows that there are no rods there. If then we can shine our alternating red and green lights upon this central spot and nowhere else we shall avoid contamination with rhodopsin, consequently any pigments measured will be cone pigments. To be sure $\frac{1}{3}$ mm^2 is rather a small area for accurate measurement nevertheless some fairly definite results have been obtained.

Now upon the fovea we can see more than one colour, so we should expect the cones there to contain more than one visual pigment. But it is easier to investigate one thing at a time than

two at once, so we shall first consider the pigment upon the fovea of colour blind subjects whom Willmer (1950) has shown can only see one colour by central vision.

2. *Foveal densitometry in the red-blind*

The type of colour blind subject investigated was an extreme form of the common red-green blindness, which is a sex-linked recessive character and hence manifest nearly entirely in males.

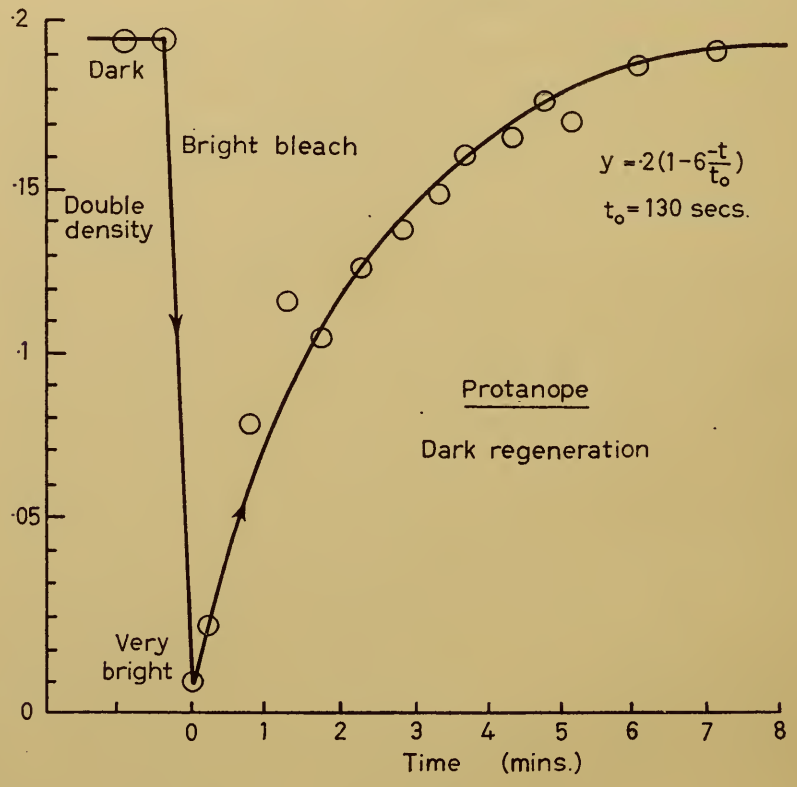

Fig. 12

Cone pigment measured on the fovea of a colour-blind subject. Effect of bleaching and regeneration (Rushton: unpublished).

The subjects, though monochromatic upon the fovea, using full vision are dichromats of the type called 'protanope'; they cannot distinguish the hue of any spectral colour from green to red, and red is seen so poorly that the yellow which they match with it has to be dimmed, nearly to blackness. They are therefore aptly called 'red-blind'. My subjects were all volunteers from the physiology classes of Cambridge University, and were remarkable

for their co-operation, intelligence and self-control in this difficult experiment where everything depends upon the absolute steadiness of the subject over a period of 10 mins. or more.

Fig. 12 shows the result of bleaching and subsequent regeneration of pigment measured upon the fovea of a protanope. The regeneration proceeds much faster than the regeneration of rhodopsin and indeed the 8 mins. required for complete recovery is about the time needed for complete recovery of cone excitability

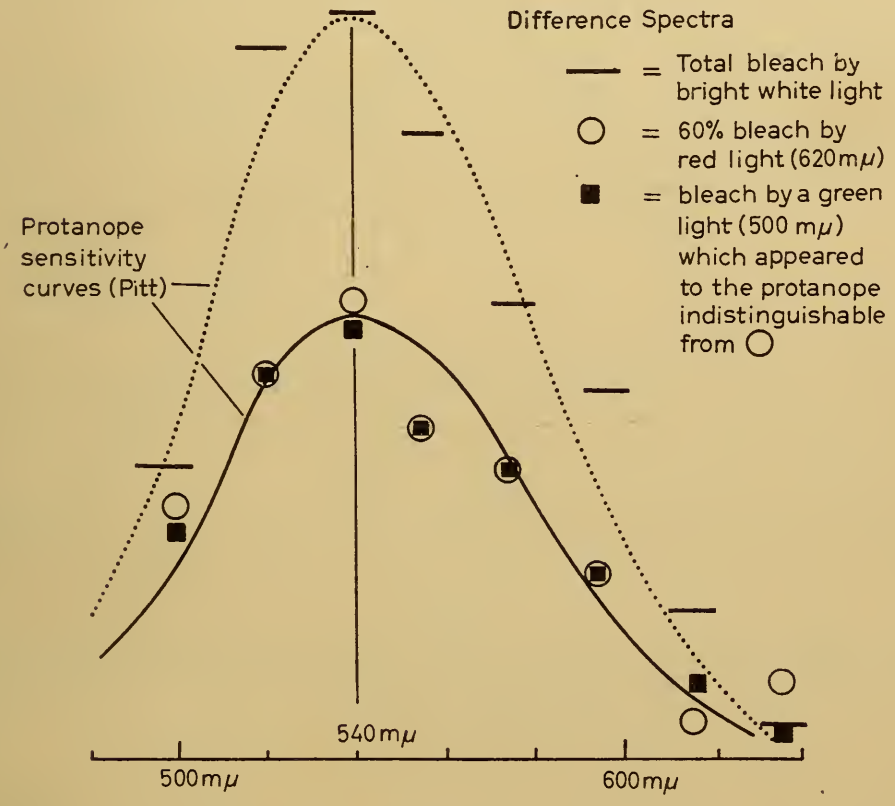

Fig. 13

Difference spectrum of the cone pigment in the protanope after half bleaching with red light (circles) or with green light (squares) or after a total bleach (lines which give the wave bands used). (Rushton: unpublished).

as seen on Fig. 11. The results of Fig. 12 are thus what might be expected of the bleaching and regeneration of one or more cone pigments.

In order to settle whether one pigment or more are present we may find the effect of partial bleaching with red or green light. The argument here runs as follows. Suppose Fig. 12 shows the

effect of bleaching two pigments, one more red-sensitive and one more green-sensitive, then, if we bleach with a red light this will affect mainly the red-sensitive whereas bleaching with a green light will affect mainly the green sensitive pigment. We detect whether a red-sensitive or a green-sensitive pigment has been chiefly bleached by obtaining the difference spectrum on the fovea

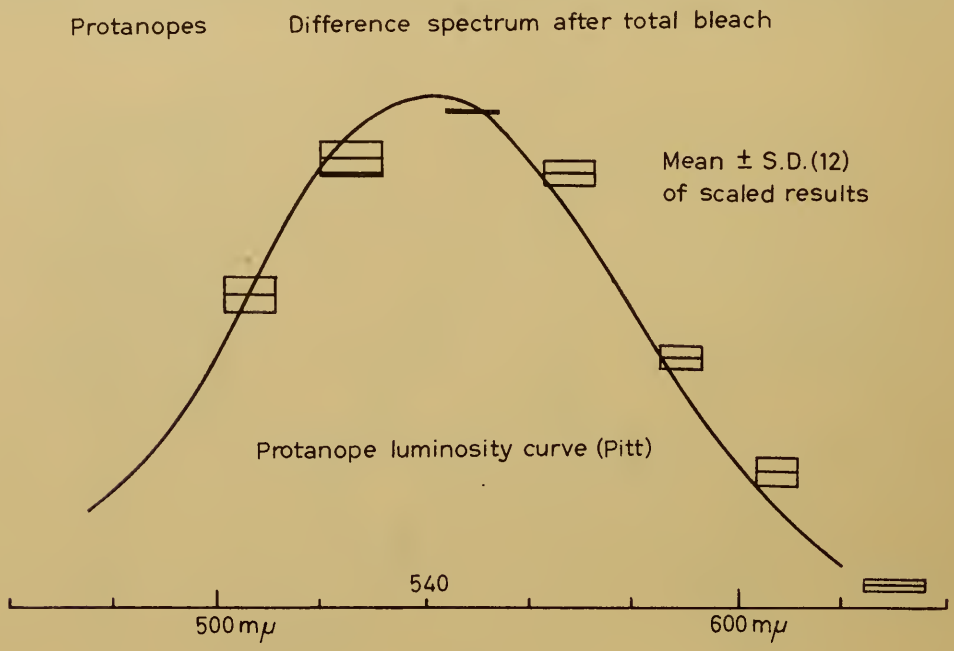

Fig. 14

Rectangles, the difference spectrum of the visual pigment of protanopes (showing wave bands used and standard duration of results). The curve is the protanope luminosity curve. (Pitt, F.H.G., 1944)

similar to Fig. 8 where it was found for rhodopsin in the periphery by using measuring lights of various wave lengths.

Fig. 13 (circles) shows the change of density at various wave lengths when about 50% of the pigment was removed by bleaching with a red light (620 mμ). The squares show the effect of a similar bleach by a blue-green light (500 mμ). The results do not support the view that the curve through the circles has its peak to the right, that through the squares has its peak to the left. Quite on the contrary the squares and circles coincide and thus show that only one pigment is present—the pigment whose difference spectrum is shown best by the total bleach (Fig. 13).

If this curve represents the absorption spectrum of the visual pigment by which the protanope sees, there should be the same relation between absorption and visibility at different wave lengths as was found for rhodopsin in Fig. 2. Fig. 14 shows the visibility curve for the protanope which Pitt found to be very constant from subject to subject, together with some rectangles which give the average difference spectrum of the foveal pigment measured at various wave lengths.

Willmer (1950) found the protanope to be monochromatic upon the fovea, and hence his vision requires but one visual pigment. Retinal densitometry has found that only one photosensitive pigment is detectable upon the fovea. That this in fact is the pigment of his vision is indicated by the coincidence seen in Fig. 14 between the spectral sensitivity for vision and the difference spectrum of this pigment measured physically.

3. *The pigments of the cones*

König (1894) and later Willmer & Wright (1945) found the *normal* fovea to be dichromatic in the sense that it is sensitive to red and green but not (much) to blue. We should therefore expect it to contain in addition to the protanope's pigment a red-sensitive pigment absent from the fovea of the red-blind. This is found to be the case by bleaching the normal fovea with a deep red light (650 mμ). Such a light appears pretty dim to the protanope, and it is not absorbed by his pigment (Fig. 14) sufficiently to bleach it. But when this light was shone into my own (normal) eye it produced the difference spectrum seen in Fig. 15 (squares). When all this pigment had been bleached away, a further total bleach with strong white light removed any other pigment present upon my fovea, and the difference spectrum (black circles, Fig. 15) is seen to correspond closely to the pigment of the protanope whose curve is drawn through my points.

This is what would be expected. It has long been known that the protanope accepts as a good match all colour matches made by the normal, which means that his condition differs from normal by the loss of one degree of freedom. By far the simplest way this can happen is for him to lose one pigment. And the objective measurements strongly indicate that this is the case.

Now that these pigments can be detected and measured it is useful to have names for them. The protanope pigment may be

called 'chlorolabe' (= green catching), the other 'erythrolabe'. But the blue pigment 'cyanolabe' which must be present in the retina to account for our blue vision has not yet been detected.

We have argued as though light reached the visual pigments unfiltered. That certainly is not the case in fowls where, as Schultze pointed out, oil droplets of various colours are placed in

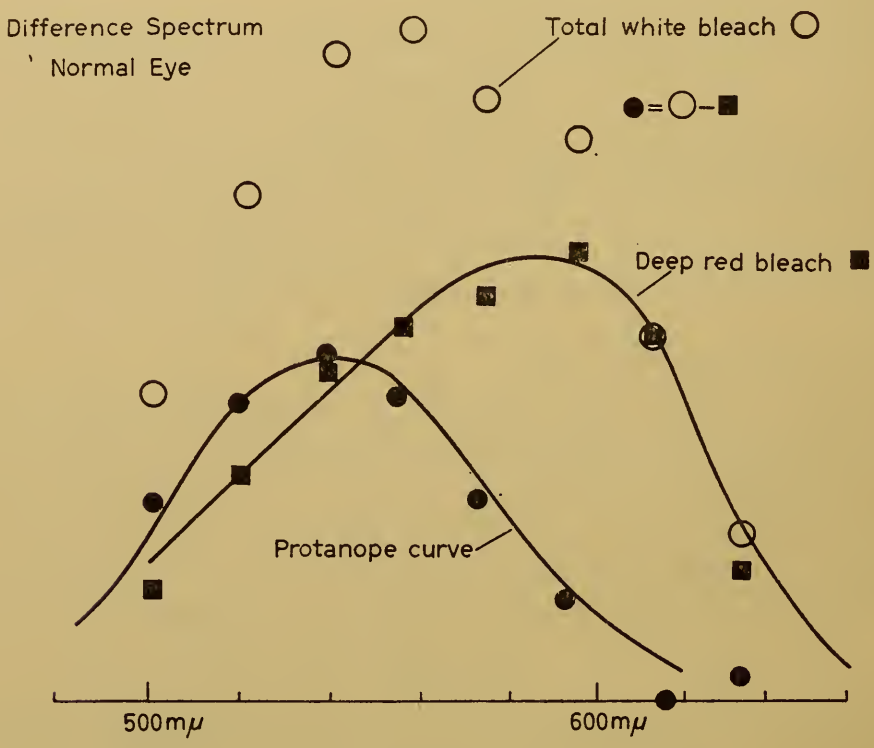

Fig. 15

Squares show difference spectrum upon bleaching with a deep red light. Circles show total bleach scaled to coincide with squares at red end. Dots show difference between circles and squares, and coincide with the curve which is the curve of the protanope, Fig. 14. (Rushton: unpublished)

the light path within the structure of the cone. It is clear that a rich colour vision could arise from the combination of several fixed stable coloured filters and one photosensitive pigment. For, each filter would in effect confer upon the pigment a new spectral sensitivity.

We have no coloured oil drops in our cones, but it has sometimes been suggested that we might have interference filters, wave guides etc., which are wave length selective and would act in

the same way. It is important to know whether what we have interpreted as the two pigments, erythrolabe and chlorolabe, is in reality a single pigment with various wave selectors interposed.

If the difference in appearance of red and green were due to some selector apparatus in front of the cone, the difference in appearance should vanish if these lights entered the retina from behind. Brindley and I tried this by leading monochromatic light through a glass rod, pushed as far as possible behind our eyes through the anaesthetized lateral fornix and pressed against the back of the internally deviated eye. A patch of light was seen whose colour was matched by light entering from in front and falling upon a contiguous patch of retina. Wave lengths less than 500 mμ were so strongly absorbed by the blood behind the eye that they could only be obtained intense enough to excite the rods, not the cones. All wave lengths greater appeared practically the same colour whether impinging upon the visual pigment from the front or the back. This makes it very improbable that colour depends upon a single visual pigment coupled with wave selectors, and erythrolabe and chlorolabe are likely to be separate pigments.

4. *The nature of protanopia*

Protanopes appear to have nothing wrong with the structure of the fovea, and their acuity is normal unless measured by red light which they cannot see. Thus they appear to have the normal cones with the normal packing. But they do not have the normal pigments in them, since erythrolabe is missing. With regard to chlorolabe, however, a striking fact is found. The quantity upon the fovea of the protanope is about twice the amount of chlorolabe in the normal fovea and is nearly equal to normal chlorolabe + erythrolabe. It thus seems very probable that those cones which in the normal contain erythrolabe, do not in the protanope stand empty and idle, but instead are filled with chlorolabe.

We have no direct information as to the biochemistry of these pigments, but from the studies of Wald, Morton and others it seems very probable that each resembles rhodopsin, and the fowl-cone pigment, idodopsin, in being the combination of a specific protein built into the structure of the photoreceptor with the 11-*cis* isomer of vitamin A_1 aldehyde. The vitamin is not specific and passes in and out of the receptors and serves both rods and cones. It is unlikely therefore to be responsible for the genetic

lack of erythrolabe. This must depend upon failure in the specific protein.

The recipes for making the special materials of our body are genetically handed down encoded in duplicate in the genes of our paired chromosomes. Our parents may mislay one copy without mishap if they give us the other—with one exception—upon the unpaired sex chromosome of the male. Here the loss of the only copy is disastrous. For a boy (whose active sex chromosome is always derived from his mother) is incapable of making the protein of erythrolabe if the chromosome she contributed lacked the recipe. But though condemned to red-blindness he need not lose all vision from half his cones. Chlorolabe he can make and this he uses to repair his loss. The 'red' cones still function but it is with chlorolabe that they respond.

IV

PIGMENTS AND COLOUR VISION

1. *Ideas and Facts*

The sight of bright colours induces strong emotion, which somehow seeps into the whole theory of colour vision, so that opinions are hotly held even by those who have never worked in the field. By contrast, most people are little concerned with the nature and mode of action (for instance) of the temperature receptors upon whose information the human body is maintained so constant that Newton and Fahrenheit could think of no better standard for the upper fixed point of the thermometer scale. Yet our failure to regulate temperature means death, whereas failure to distinguish between red and green is so trivial in human affairs that it has probably existed in some 5% of males since the beginning of history without being even detected until the enlargement of scientific curiosity 200 years ago brought this and a multitude of other oddities under inspection.

The general emotional attitude in colour vision is similar to that often seen in politics and religion—a strong partisanship towards particular views, and crusading tendencies. My own views, of course, are both moderate and reasonable—but so say all extremists. You must be judge. Do my views coincide with yours? That is the touchstone.

There are two ways in which the facts of observation relate to our ideas of mechanism. Some ideas are seductive because in terms of them a collection of facts falls into a relatively simple and coherent pattern. Some facts are compulsive because they are logically incompatible with certain ideas. It is important to remember that an idea, however seductive, is logically untenable if excluded by a compulsive fact. We each have our individual views as to what constitutes seduction—and colour vision is full of it—but there should be little disagreement upon the logic of incompatibility, or reluctance to discard concepts when clearly incompatible with compulsive fact.

2. *The Trichromacy of colour matching*

The central observation of human colour vision is that every uniform coloured field may be matched uniquely by a mixture of three 'primary' colours. This means that if the original colour is suddenly replaced by the mixture, the observer will not be aware that the substitution has occurred if (and only if) the three primaries of the mixture stand in the correct proportion. A more usual way of performing the comparison is to place the two fields to be matched side by side, and to adjust the mixture so that the fields are judged identical. The proportions required for the match are in practice the same by both methods in uniform conditions.

Now the appearance of any given light may change greatly as a result of adaptation, contrast etc., for instance a yellow light may appear rather red or rather green following adaptation to strong green or red light respectively. But, though the hue of the yellow light is thus altered, the trichromatic mixture which matches it is altered in exactly the same way, so the validity of the match is much more stable than is the appearance of the colour. This important generalization, that any two lights which appear identical in one condition will appear identical in another condition was enunciated by Grassmann (1853) a century ago, and is valid in all conditions except with lights of dazzling intensity.

Let us consider what this means in terms of visual pigments. The fovea in the protanope, as we have seen, is monochromatic and only one visual pigment, chlorolabe, can be found there. The normal fovea is dichromatic and both chlorolabe and erythrolabe are detected. But the full range of vision is trichromatic so one more independent chromatic input is necessary, namely a third pigment which is not present upon the fovea to any large extent. Could this be the rhodopsin of the rods?

Rods constitute 95% of all the human photoreceptors so it seems wasteful for them to do nothing in daylight. A seductive hypothesis is that they are the blue receptors of colour vision. In support of this there is a strong psychological association between blueness and twilight vision, and blue receptors like rods, are few upon the fovea, and have poor acuity and a large increment threshold (Fechner fraction). But this seductive hypothesis is excluded by the following compulsive fact, discovered by Stiles.

Cones at any given point in the retina are excited by light entering through the centre of the dilated pupil far more strongly

than they are by light entering through its periphery. Since the latter rays fall upon the cones obliquely, the phenomenon is called the retinal directional (or Stiles-Crawford) effect. Now Stiles (1939) has shown that cones exhibit the retinal directional effect but that rods do not; the blue receptors do, and hence cannot be rods.

Though this fact shows that blue receptors cannot be rods they might still be cones containing rhodopsin. But if there are only three pigments in colour vision, rhodopsin cannot be one of them, whether situated in rods or cones. For two metameric matches (i.e. fields which appear to the eye identical though in fact of different spectral composition) would not in daylight appear the same unless they affected *each* of the three visual pigments identically. Thus if one of the pigments is rhodopsin, every pair of metameric matches must affect rhodopsin equally and hence (after suitable attenuation) be also a good match by twilight vision. But matches valid by daylight may be very far from good matches by twilight. Hence rhodopsin cannot be one of the three cone pigments, and there must be a blue-sensitive pigment *cyanolabe* which has not yet been objectively detected.

Though it seems impossible that there can be less than three cone pigments, it is conceivable that there could be more. The unrestricted consequence of this would be four (or more) rather than three dimensions of colour in daylight vision. This is an advantage which we do not possess, so for every cone pigment we postulate in excess of three we must also postulate a restricting condition which exactly cancels all the advantages which such an enlarged colour system could bring. This seems to me so perverse a concept, that I strongly favour the simpler alternative, namely that we have only three cone pigments and use these independently to give us the three dimensions of colour which we certainly experience. But if this is accepted, Grassmann's law of the stability of metameric matches follows at once. For colours which match are those which are absorbed equally by each of the three pigments, and since the absorption spectrum of each pigment remains unchanged in shape by visual procedures, colours which matched before will match after or during such procedures. But the appearance of the colours depends upon nerve interactions and may change very greatly.

3. *The polychromacy of colour naming*

The trichromacy of colour matching has led some to speak of 'the three primary colours', a statement to which many have objected because they could see red, yellow, green and blue, none of which looked to be compounded from mixtures of the others. But between coloured lights and their appearance stand not only the three pigments but a nervous apparatus of very formidable complexity. From the former we can predict the trichromacy of colour matching, from the latter we can predict almost nothing—and hence must be prepared to accept almost anything. If Hering could see four 'primary' colours and Newton seven, there is not much more that can usefully be said.

People with normal sight are agreed about the parts of the spectrum whose lights are called red, yellow, green and blue, and these are recognized without a reference standard. For instance, upon waking at night a single red light in the darkness is confidently recognized as red. Over-confidently, for we are extremely reluctant to realize how fallible may be our memory and how mutable our sensations. In our sensations all estimates of intensity are relative and enormously subject to adaptation. Our estimates of quality are also relative when this depends upon the intensity ratios of various sensory inputs; it is only absolute when it depends upon the identity of the particular nerves involved, and is independent of the intensity factor (*e.g.* musical pitch, or cutaneous localization).

The appearance of colour clearly depends largely upon the relative intensity signalled by the three types of cone, hence it might be expected to adapt in the way it does. Yet we are generally surprised by the 'unnatural' appearance of objects immediately after removing coloured spectacles which have been worn for a little time, or by the complementary negative after-images of bright fields.

More striking still than the transient effects of successive contrast are the maintained phenomena of simultaneous contrast. These have been known and studied for over a century, but have been brought to a new order of prominence by the remarkable recent demonstrations of Land (1959) where two coincident pictures projected upon a white screen, one an ordinary black-white photographic positive, the other a somewhat similar black-white positive but projected through a red filter, are seen as

a picture in a striking range of colour including very bright green.

The explanation of the phenomena of simultaneous contrast must rest largely upon interaction between nerves from neighbouring regions of the retina. Eye movements will convert spatial variation over the retina into temporal variation in each locality. But Land has shown his demonstration with a flash of light so brief that no eye movement could take place, and the nerve interaction between regions must have occurred during the actual generation of the sensory signal. The fact that observers of Land's demonstration will name in the picture a range of colours far outside that obtainable by all mixtures of the two projection lights in conditions of an ordinary colour match, has led some writers to claim that the trichromacy of colour mixtures is wrong. What is wrong, however, is merely their understanding of the statement of trichromacy. This has nothing to do with colour naming, but is concerned with the sudden replacement of one coloured light by a mixture so identical that the subject cannot tell which is which. Such substitution cannot be achieved for all colours without the use of three fixed 'primaries' in the substituted mixture. But three are sufficient. If each small region of Land's picture were analysed by substituting in that very place a metameric red, green, blue mixture, we should doubtless get out the dichromacy that Land put in. Every match would need quantities r, g, b, of three primaries, and there would be found a fixed linear relation between r, g and b thereby reducing the degrees of freedom from 3 to 2. On the other hand with unrestricted range of r, g, b it would be possible to introduce a whole dimension of colour that Land could not achieve.

I say 'doubtless' for the experiment has not to my knowledge been performed, but I do not much doubt the result. It is a particular case of Grassmann's law which has never yet known an exception, and which itself is a consequence of the three pigments of the cones.*

4. *The Nature of the three cone pigments*

Unfortunately the identity of the three pigments cannot yet be regarded as settled. If we knew their spectral sensitivity we should know the relative absorption of each for every wave length of

*I have now performed this experiment and confirmed the anticipated result. (1961) *Nature 189*, 440.

light, and could state in what proportion certain 'primary' red, green and blue lights would have to be mixed to give a metameric match. Now in fact the proportions of three primaries required to match each wave length of the visible spectrum are known with good precision from the careful work of several authorities who agree, and it might be hoped that from this the spectral absorption of the three pigments could be extracted. Unfortunately the process of 'unmixing' is far from unique and we may obtain an infinite number of solutions all different and (mathematically) equally acceptable. In order to proceed further we need additional information, and that is either hard to obtain with the necessary precision or is doubtful in its application to the absorption spectrum of the visual pigments. Here four approaches will briefly be mentioned.

(a) *Measurements of pigment by retinal densitometry*

The objective measurement of pigments is in principle most free from misinterpretation. Unfortunately the accuracy is very far inferior to that of sensory observation. And difficulties with stray light and with the analysis of two pigments simultaneously, severely limit quantitative results at present. Moreover, the blue-sensitive pigment has not yet even been detected.

(b) *The spectral sensitivity of dichromats*

If we assume that the fovea of a protanope contains only the visual pigment chlorolabe, and the fovea of a deuteranope only erythrolabe, then the foveal spectral sensitivity of these dichromats will give us the absorption spectrum of the two pigments. This suggestion is an old one and has often been contested, but densitometry measurements appear to support it. Lights of different wave lengths which the protanope judges identical are found to be equivalent in their power to bleach chlorolabe on his fovea (measured at 540 mμ). Similarly lights judged identical by the deuteranope are equivalent in bleaching his erythrolabe (measured at 610 mμ).

The agreement is shown in Fig. 16. In A the circles show Willmer's (1950) log sensitivity results for various wave lengths measured on the fovea of the protanope, and the vertical lines give the log energies of lights which bleach chlorolabe equally.

In B the circles show similar results with the deuteranope and the lines represent the action spectrum of erythrolabe.

(c) *Artificial Monochromacies after adaptation to very bright coloured lights*

Brindley (1953) has found immediately following adaptation to intense violet and then to intense red light, that the whole spectrum from 480 to 620 mμ appeared of the same colour so that it was possible to match each wave length with a fixed yellow by varying intensity only. If we suppose that after this treatment the nerves from the green receptors were the only ones in a state to transmit messages, then the relation obtained in this matching experiment would give the spectral sensitivity of the green receptors. The crosses of Fig. 16A show what this spectral sensitivity is. It agrees fairly well with the circles and vertical lines, and supports the view that green receptors contain chlorolabe only.

A similar experiment following adaptation to violet and intense blue-green light permitted yellow to be matched with any light from 500 to 700 mμ in wave length. The spectral sensitivity in this case was not the same as in the former experiment, and is plotted as crosses in Fig. 16B. The results agree with the view that the only effective receptors were the red cones and that these contain erythrolabe only.

(d) *Increment thresholds*

If a flashing light is superimposed upon a more or less bright background it will not be seen unless it is rather bright itself. But it is more easily seen if the colour of the flash is very different from that of the background. What is the relation between wave length and the intensity of flash and background in these circumstances? From a very extensive study Stiles (1949) has reached results of great formal simplicity.

His chief conclusions are that cone increment thresholds depend upon independent colour mechanisms with three spectral sensitivities. If, for instance, the flash is red, it will excite the red mechanism which will be depressed by the background field to the extent that this also excites the red mechanism. The extent to which other mechanisms are stimulated is irrelevant for the red increment threshold.

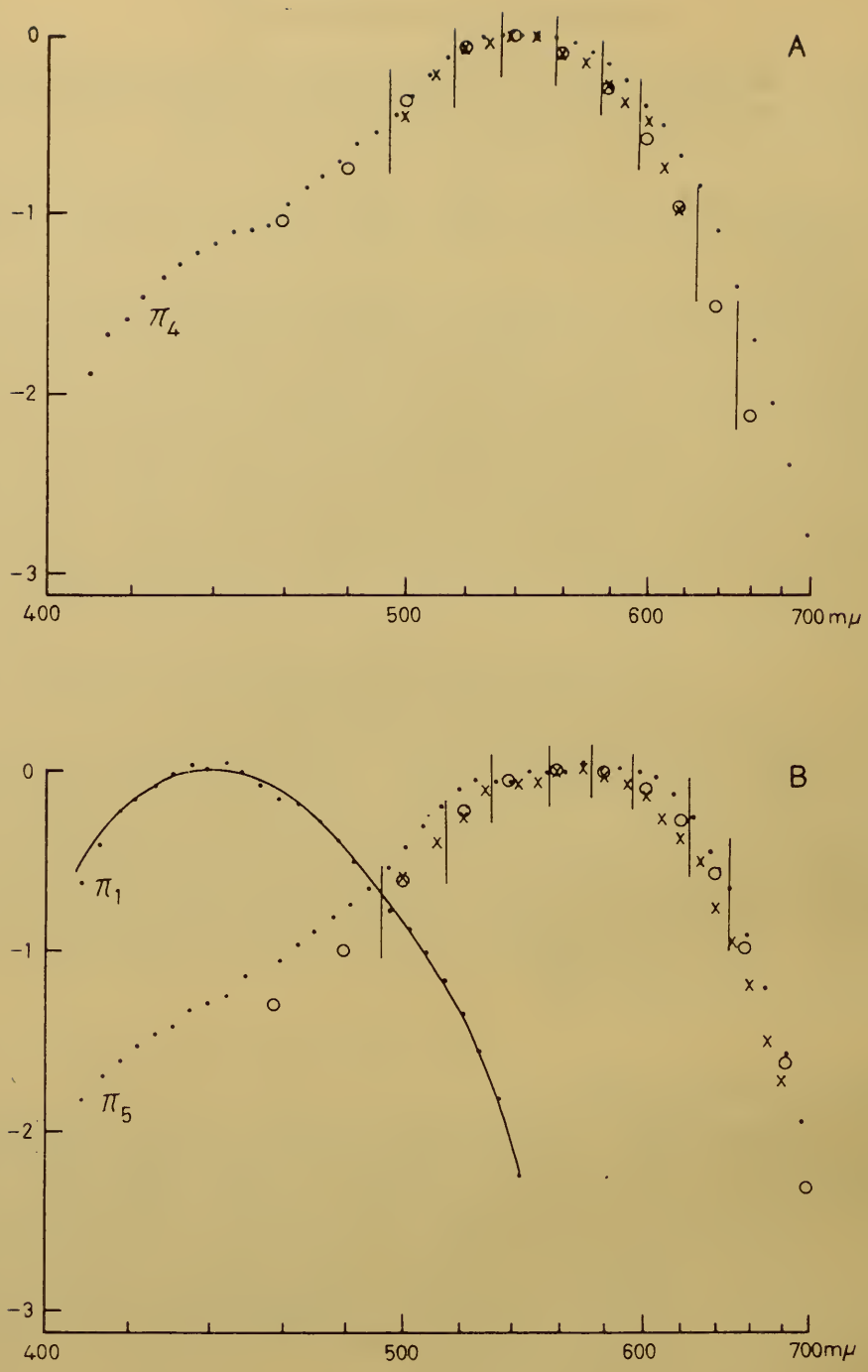

Fig. 16

Log spectral sensitivity curves plotted against wave frequency. Vertical lines: action spectral. A, Chlorolabe, B, erythrolabe (Rushton). Circles: spectral sensitivity of the fovea of A, protanopes; B, deuteranopes (Willmer 1950). Crosses: artificial monochromacy, A, green; B, red (Brindley 1953). Dots: increment threshold mechanisms, A, the green mechanism π_4; B the red mechanism π_5 and blue mechanisms π_1 and π_3. Curve: the blue mechanism calculated from colour matching. (Stiles 1953).

The spectral sensitivities of Stiles' mechanisms are shown in Fig. 16, the green π_4 by dots in A, the red π_5 and the blue π_1 by dots in B. The results fit the other curves moderately well and hence are in general agreement with the view that π_4 catches quanta by means of chlorolabe, and π_5 by erythrolabe. Does π_1 catch quanta by means of cyanolabe?

We have much less information about this pigment than the others, for retinal densitometry has not detected any and indeed there is probably little of it on the fovea. Brindley's (1953) violet monochromacy differed from his green and red monochromacies in that intensity discrimination was so very poor that no spectral sensitivity could be determined. And colour blindness does not give us much help with the blue mechanism unless the extremely rare "blue cone monochromats" described by the Blackwells do in fact have normal blue cones and no others.

Stiles (1953), however, has obtained an accurate sensitivity curve for his blue mechanism (more precisely his two similar blue mechanisms π_1 and π_3) shown plotted as dots in Fig. 16B. At 530 mμ the sensitivity has dropped by 1.7 log units *i.e.* to 2% of the peak value, consequently if π_1 represents the absorption of cyanolabe, that pigment will absorb a negligible amount of light at wave lengths 530 mμ or greater. But that will allow us to derive the absorption spectrum of cyanolabe from the well-known and accurate colour mixture results using primaries 650, 530 and 460 mμ, since only the blue primary will contribute to absorption by cyanolabe. The continunous curve of Fig. 16B is that calculated by Stiles (1953) in this way (with some refinements).

It must be emphasized that the good fit of curve and dots in no way represents two manipulations of the same kind of measurement to produce the same results. The *curve* is derived from the identical appearance of two physically different fields and depends upon the visual pigments involved, but not at all upon the way that these may be mixed in various types of cones or upon the complexity of nerve interconnections. The dots show the relation between intensity and wave length of a background which will just permit a fixed blue flash to be detected by the brain. This would certainly depend upon the way that cyanolabe and other pigments were mixed in the cones concerned and upon summations and inhibitions between 'blue' cone pathways and those from other cones. The good fit therefore between curve and

triangles is not tautology but evidence that π_1 catches quanta by means of cyanolabe, just as π_4 catches them by chlorolabe and π_5 by erythrolabe.

CONCLUSION

In colour vision it is not easy to be original without being outrageous, and I claim no originality in what I have said. To the work of others, which for the most part is well-known, I have ventured to add some rather inaccurate, but objective measurements of my own. As for my conclusion that there are three visual pigments each connected to a separate cone output, it may be found in Thomas Young's Bakerian lecture to the Royal Society in 1802.

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